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1 Title of invention

1 Please give the title of the invention: VACCINE COMPOSITIONS

2 Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

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Country *(and State of incorporation, if appropriate)*: NETHERLANDS

2b If you are applying as an individual or one of a partnership please give in full:

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Agent's address: St. Georges House
6 Yattendon Road
Horley
Surrey

Postcode: RH6 7BS

Agent's ADP number: 05880273001 ✓

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6 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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Continuation sheets for this Patents Form 1/77	0		
Claim(s)	3	Description	12
Abstract	1	Drawing(s)	2

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VACCINE COMPOSITIONS

The present invention relates to vaccine compositions for delivery to mucosal surfaces, and to a method of inducing, in a mammal, an immune response to an antigen or a mixture of antigens by delivering the antigen or mixture of antigens to a mucosal surface of the mammal.

More particularly, the present invention relates to vaccine compositions for inoculating a mammal such as a human against B.pertussis infections.

The majority of infectious diseases are initiated by contact with a mucosal surface. The infecting agent may remain at or within the mucous membranes during the course of the infection or may penetrate into the body and localise at other sites. The importance of the mucous membranes in the first line of defence against infectious disease can be gleaned from the fact that 90% of the lymphocytes of the body underlie such surfaces. Priming

mucosal surfaces by immunisation so that they respond vigorously and effectively control pathogenic organisms they encounter would be advantageous. Unfortunately traditional immunisation regimes are ineffective at eliciting mucosal responses. The systemic and local (mucosal) immune systems appear to be compartmentalised and in general do not impinge on one another; that is parenteral immunisation with non-living vaccines stimulates mucosal immune responses weakly if at all. Mucosal immunisation (oral or intranasal) can evoke serum antibodies but this is usually less effective than parenteral immunisation. The immunocytes of the different mucous membranes form a vast intercommunicating network, termed the common mucosal immune system, such that topical immunisation of one surface (e.g. the gastrointestinal tract) may lead to an immune response at that surface and also distance surfaces such as the respiratory tract.

Manclark and Shahin (US Patent application number 07/532 327, filed 5.6.90 - available through the US Department of Commerce, National Technical Information Service, Springfield, VA 22161, USA) - have described the intranasal and intraduodenal administration of filamentous hemagglutinin (FHA) obtained from Bordetella pertussis and have illustrated that FHA is an effective mucosal immunogen. Manclark and Shahin speculated in USSN 07/532,327 that the 69-kD outer membrane protein (P69) of B.pertussis would also be an effective mucosal immunogen, but presented no experimental data to show that this was

the case.

The fact that there are very few mucosal vaccines commercially available indicates that there are problems with developing such vaccines Many non-living soluble antigens, particularly those used traditionally by immunologists, such as ovalbumin (OVA) and Keyhole Limpet Haemocyanin (KLH), are poor mucosal immunogens. Large doses of such antigens are necessary to induce any responses but large doses can also cause tolerance in the individual to subsequent parenteral exposure to antigen, a condition known as oral tolerance. Some microbial components such as the cholera toxin (CT) or E.coli heat-labile toxin (LT) or the non-toxic binding portions of these toxins (CT-B and LT-B) have been found to be potent mucosal immunogens eliciting strong secretary and circulating antibodies, but the reason why such molecules are good mucosal immunogens has not yet been fully elucidated. One property that may be important is the ability of these molecules to bind to mucosal epithelial cells via certain surface receptors, although it has been found in studies by others that there is not necessarily a correlation between the ability of an antigen to bind to eucaryotic cells and its mucosal immunogenicity.

Thus, as far as we are aware, there is currently no way of predicting with any certainty whether a given antigen will possess good mucosal immunogenicity.

It has now been found that a particular mutant form of p rtussis toxin has good immunogenic activity when given

via the intranasal route. In particular, it has been found that a non-toxic double mutant form of pertussis toxin, more particularly the non-toxic double mutant form wherein the glutamic acid residue at location 129 in the S1 sub-unit has been substituted by glycine and the arginine 9 amino acid residue has been substituted by lysine, when given intranasally gives good protection against B. pertussis challenge.

In a first aspect, the invention provides the use of an antigen for the manufacture of a vaccine composition for intranasal administration to induce an immune response against B. pertussis infection, characterised in that the antigen is a non-toxic double mutant form of pertussis toxin.

The invention also provides a vaccine composition adapted for intranasal administration, the vaccine composition comprising a pertussis toxin mutant as hereinbefore defined and a pharmaceutically acceptable carrier.

Preferably the non-toxic double mutant form of pertussis toxin is one in which the Glu129 amino acid in the S1 sub-unit has been substituted by another amino acid.

Particular examples of non-toxic double mutant pertussis toxins suitable for use in the present invention are those disclosed in European patent application EP-A-0462534 (Sclavo SpA). A preferred non-toxic double mutant toxin is the mutant described in example 1 of EP-A-0462534, in which the arginine 9 residue has been substituted by

lysine, and the glutamic acid 129 residue has been substituted by glycine. This mutant is referred to hereinafter as PT 9K/129G.

In a particular embodiment, the pertussis toxin mutant may be combined with one or more other pertussis antigens, for example filamentous haemagglutinin (FHA), and/or the 69 kilodalton outer membrane protein (P69 - also known as pertactin) from B. pertussis.

In another aspect the invention, provides a method of immunising a host such as a mammal, (e.g. human) against infection, which method comprises administering an effective amount of the antigen or a mixture of antigens as hereinbefore defined, directly to a mucosal surface in the host to induce in said mucosal surface an immune response to each said antigen.

The compositions typically are formulated as an aqueous solution for administration as an aerosol or nasal drops, or as a dry powder, e.g. for inhalation.

Compositions for administration as nasal drops may contain one or more excipients of the type usually included in such compositions, for example preservatives, viscosity adjusting agents, tonicity adjusting agents, buffering agents and the like. The antigen or mixture of antigens typically is selected such that it is non-toxic to a recipient thereof at concentrations employed to elicit an immune response.

In order to enhance the mucosal immunogenicity of the mutant pertussis toxin and optionally the oth r antigens,

they may be incorporated into appropriate carriers. Immunogenicity may also be enhanced by incorporating appropriate mucosal adjuvants such as cholera toxin or E.coli heat-labile toxin, genetically detoxified variants thereof or their binding (B) sub-units in the vaccine.

The vaccine composition may in addition, contain one or more further mucosally immunogenically active antigens.

The P.69 outer membrane protein of B.pertussis is a protein of approximately 61 KD molecular weight; see A.J.Makoff et al, "Protective surface antigen P.69 of Bordetella pertussis: its characteristics and very high level expression in Escherichia coli", Bio-Technology, 8, 1030 (1990).

It can be prepared and isolated according to the method disclosed in P.Novotny et al: The Journal of Infectious Diseases, 164, 114 (1991), or recombinant material prepared from E.coli by the method given in the article by A.J.Makoff et al referred to above. It can bind to eukaryotic cells.

Purified B.pertussis filamentous haemagglutinin usually contains polypeptides of differing molecular weight ranging from 98-220 KD, and can be isolated and purified from cell culture supernatants of B.pertussis, for example as described in the article by P. Novotny et al referred to above. The filamentous haemagglutinin is able to bind to eukaryotic cells and cause haemaglutination of sheep erythrocytes.

The antigenic molecules of the present invention can

be prepared by isolation and purification from the organisms in which they occur naturally, or they may be prepared by recombinant techniques and expressed in a suitable host such as E.coli in known manner. When prepared by a recombinant method or by synthesis, one or more insertions, deletions, inversions or substitutions of the amino acids constituting the peptide may be made.

The aforementioned antigens are preferably used in the substantially pure state. The quantity of the mixture of antigens administered will depend, in part, upon the purity of the individual antigens. Thus, for a substantially pure form of the non-toxic double mutant pertussis toxin, or the P.69 outer membrane protein, a dose in the range from about 1-100 microgrammes/dose typically would be administered to a human, the actual amount depending on the immunogenicity of the preparation in humans when applied to mucosal surfaces.

For a substantially pure form of the B.pertussis filamentous haemagglutonin, a typical dose range would be of the order given above in, respect of the mutant pertussis toxin or P.69 protein. In a typical immunisation regime employing the antigenic preparations of the present invention, the vaccine may be administered in several doses (eg 1-4), each dose containing 1-100 microgrammes of each antigen. The immunisation regime may involve immunisation purely by the mucosal route, or a combination of mucosal and parenteral immunisation. The dosage will in general depend upon the immmunogenicity of the different antigens

when applied to the respiratory tract of animals.

The invention will now be illustrated in greater detail by reference to the specific embodiments described in the following examples.

The examples are intended to be purely illustrative of the invention and are not intended to limit its scope in any way.

EXAMPLE 1

Mice were immunised intranasally three times with B. pertussis PT-9K/129G mutant obtained from Sclavo (4.4 microgrammes per dose) or ovalbumin (10 microgrammes per dose) the second and third doses being administered at 28 days and 150 days respectively. Elispot analyses (see below) were performed after the second and third doses to determine the immune response in the lungs of the mice. The antibody responses in the lungs taken seven days after the second dose, and 5 days after the third dose are shown in Table 1 below. From the results it can be seen that the immune response to intranasally administration of mutant pertussis toxin PT-9K/129G was significantly better than the response stimulated by ovalbumin (OVA).

TABLE 1

	Serum Response	Lung ELISPOT - 2nd dose (ASC/10⁶ lymphocytes)			Lung ELISPOT - 3nd dose (ASC/10⁶ lymphocytes)		
		IgG	IgA	IgM	IgG	IgA	IgM
PT9K/129G	2500	2706	<50	39	4423	1231	115
OVA	<50	<40	<40	40	49	<49	244

EXAMPLE 2

Intranasal immunisation with a combination of FHA, P69, and pertussis toxin mutant PT 9K/129G

BALB/c mice were immunised intranasally with 10 micrograms of each of the above antigens, 36 and 14 days prior to aerosol challenge with B.pertussis BBC 26. Adult (6-8 weeks) mice were anaesthetised with metathane and the antigen solution was added to the external nares of the mice as they recovered consciousness. Antigen was taken into the respiratory tract by inhalation.

The results obtained from the analysis of colony forming units of B.pertussis in the lungs are shown in Figure 1. For comparison purposes the corresponding figures obtained from subcutaneous immunisation are also shown. As can be seen, immunisation via the nasal route gave results broadly equivalent to those obtained by the subcutaneous route.

The results of the analysis of the bacterial counts of B.pertussis retrieved from nasal lavage are shown in Figure 2. Again, the figures obtained by subcutaneous administration are shown by way of comparison. From the figures, it can be seen that immunisation by the subcutaneous route gave rise to a slightly greater reduction in bacterial numbers than was obtained by intranasal administration up to about the 10 day point, but from about 10 days onwards, the bacterial numbers showed a greater reduction in animals immunised via the intranasal route.

MATERIALS AND METHODS

AEROSOL CHALLENGE WITH B.PERTUSSIS

Mice were placed in cages on a rotating carousel in a plastic exposure chamber as described in P.Novotny et al. Development for Biological Standards, 61, 27 91985). A bacterial suspension in PBS was prepared from 2-to 3-day old cultures of B.pertussis BBC26 grown on CW blood agar plates. The mice were exposed to an aerosol (generated from the bacterial suspension) of 2 x 109 Colony-forming units (CFU) in PBS by a Turret mouthpiece tubing operated by a System 22 CR60 high-glow compressor (Medic-Aid), Pagham, West Sussex, UK) giving a very fine mist at a dynamic flow of 8.5 litres/min. The generated mist was drawn through a chamber by a vacuum pump at a passage of ca.12L of air per mist mixture per min, which maintained 70% relative humidity in the chamber. The exposure to aerosol lasted 30 min; a period of 10 min then allowed the chamber to clear.

The course of the infection was assessed by performing counts of viable bacteria in lungs. Groups of four mice were removed at intervals and killed by cervical dislocation, and their lungs were aseptically removed and homogenised in a Potter-Elvehjem homogenizer with 2ml of PBS. Dilutions of the homogenate were spotted onto Cohen-Wheeler (CW) blood agar plates and the number of CFU was determined f r each set of lungs.

ANTIGENS

The outer membrane protein of B.pertussis, P69, was synthesised intracellularly in E.coli and purified as described in A.J.Makoff et al, Bio/Technology 8, 1030 (1990). Filamentous haemagglutinin (FHA) was provided by SKB under an exchange of reagents agreement. Antigens were diluted in PBS immediately prior to immunisation.

ELSIPOT Assay for specific antibody secreting cells (ASC) in murine lungs.

Local antibody production in the murine lung was determined using the ELISPOT technique. Lymphocytes were isolated from murine lungs as follows: Lungs were washed briefly in PBS to remove traces of blood and then were finely chopped with a scalpel blade. 1ml of PBS containing 10mM MgCl2, 0.5U/ml collagenase A (Boehringer Mannheim, Lewes, UK) and 0.25mg/ml DNase 1 (Boehringer) was added for each pair of lungs and incubated at 370C with gentle agitation for 45 min. The mixture was then passed through a 40 gauge mesh. Lumps were pressed through the mesh with the plunger from a 5ml syringe. The cell suspension was placed in a centrifuge tube and allowed to stand for several minutes to allow large debris to settle. The supernatant was removed and the cells were pelleted and washed several times. Red cells and non-viable cells were removed by centrifugation on a Ficol-Isopaque gradient (LSM,Flow Laboratories Ltd, Herts, UK). After washing cell viability was d termined by Trypan Blue exclusion. Cells

were finally suspended in RPMI1640 complete Medium (10% foetal calf serum, penicillin 100IU/ml, streptomycin 100 g/ml, L-glutamine 2mm; Flow).

The ELSIPOT assay was performed as follows. Briefly, 24-well tissue culture plates (Costar) were coated overnight with P.69, FHA or OVA (0.5ml of 1 g.ml in PBA) after washing and blocking 0.5ml volumes of dilutions of the lymphocyte suspension in complete RPMI 1640 were added to the wells and incubated at 370C/10% CO2 for 3h. After washing goat anti-mouse IgG, A or M (1/1000, Sigma) and rabbit anti-goat IgG-alkaline phosphatase (1/1000, Sigma) were added sequentially. Finally, substrate solution (0.5 l of 1mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in 2-amino-2-methyl-1,3-propanediol (AMP) buffer, Sigma) was added and plates were incubated until blue spots were visible under low power microscopy.

CLAIMS

1. The use of an antigen which is a non-toxic double mutant form of pertussis toxin for the manufacture of a vaccine composition for intranasal administration to induce an immune response against B.pertussis infection.

2. The use according to Claim 1 wherein the non-toxic double mutant form of pertussis toxin is one in which the glutamic acid 129 amino acid in the S1 sub unit has been substituted by another amino acid.

3. The use according to Claim 2 wherein the glutamic acid 129 amino acid has been substituted by glycine.

4. The use according to any one of the preceding Claims wherein the arginine 9 amino acid has been substituted.

5. The use according to Claim 4 wherein the arginine 9 amino acid has been substituted by lysine.

6. The use according to any one of the preceding Claims wherein the vaccine composition contains one or more other pertussis antigens selected from filamentous haemagglutinin (FHA) and the P69 outer membrane (P69).

7. The use according to Claim 6 wherein the vaccine composition contains both FHA and P69.

8. A vaccine composition adapted for intranasal administration, the vaccine composition comprising a non-toxic double mutant form of pertussis toxin, and a pharmaceutically acceptable carrier.

9. A vaccine composition according to Claim 8 wherein the non-toxic double mutant form of pertussis toxin is one in which the glutamic acid 129 amino acid in the S1 sub unit has been substituted by another amino acid.

10. A vaccine composition according to Claim 9 wherein the glutamic acid 129 amino acid has been substituted by glycine.

11. A vaccine composition according to any one of Claims 8 to 10 wherein the arginine 9 amino acid has been substituted.

12. A vaccine composition according to Claim 11 wherein the arginine 9 amino acid has been substituted by lysine.

13. A vaccine composition according to any one of Claims 8 to 12 which contains one or more other pertussis antigens selected from filamentous haemagglutinin

(FHA) and the P69 outer membrane (P69).

14. A vaccine composition according to Claim 13 which contains both FHA and P69.

15. A vaccine composition according to any one of Claims 8 to 14 in the form of nasal drops or a nasal spray.

16. A vaccine composition according to any one of Claims 8 to 15 packaged in a container adapted to dispense a metered dose of the composition in spray or drop form.

17. A method of immunising a host such as a mammal (e.g. human) against B.pertussis infection, which method comprises administering to the host intranasally an effective amount of a composition as defined in any one of Claims 8 to 16.

Figure(s) to accompany [illegible]

ABSTRACT

VACCINE COMPOSITIONS

The invention provides the use of an antigen which is a non-toxic double mutant form of pertussis toxin for the manufacture of a vaccine composition for intranasal administration to induce an immune response against B.pertussis infection. The invention further provides a vaccine composition adapted for intranasal administration, the vaccine composition comprising a non-toxic double mutant form of pertussis toxin, and a pharmaceutically acceptable carrier. The non-toxic double mutant form of pertussis toxin is preferably one in which the glutamic acid 129 amino acid in the S1 sub unit has been substituted by glycine and the arginine 9 amino acid has been substituted by lysine.

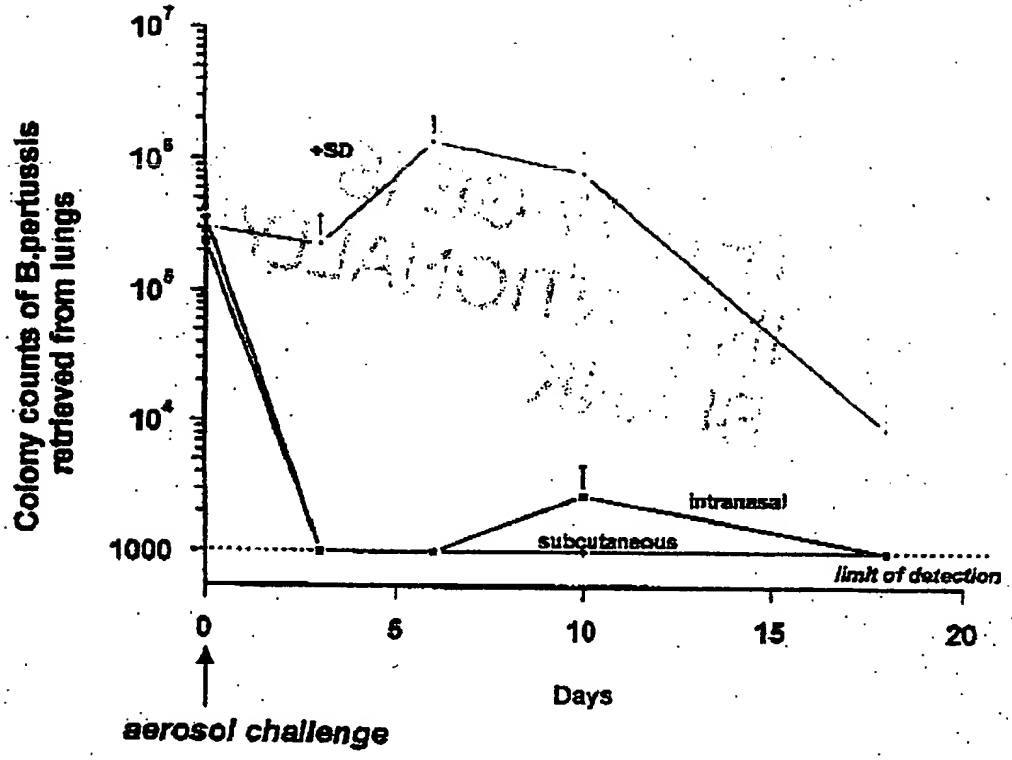

FIGURE 1

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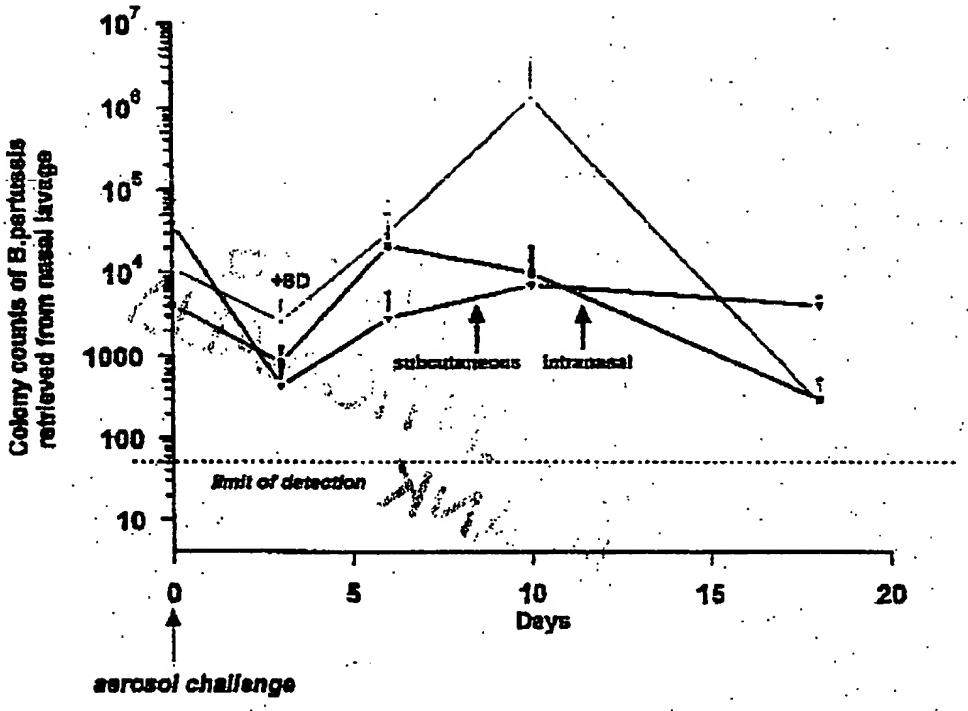

FIGURE 2

Fry Health + Spence

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